

Project of ample dissemination of the results of a joint project between the Faculty of Medicine of Ribeirão Preto and the Institute of Physics of São Carlos, University of São Paulo

Projeto amplo de divulgação de resultados de projeto conjunto entre a Faculdade de Medicina de Ribeirão Preto e o Instituto de Física de São Carlos da Universidade de São Paulo

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The liver can be the target of various diseases, with resulting physiological changes that compromise the general functioning of the organism, with a possible fatal outcome for the patient. Hepatic diseases of current high clinical interest are: hepatocellular carcinoma, cirrhosis, cholestasis, and steatosis. In cases of irreversible involvement of the organ, a liver transplant may be the only treatment alternative.

In a reasonable number of cases, the diagnosis of hepatic injuries is made by histopathological analysis of a tissue sample collected with a biopsy after tissue processing. The cellular and tissue changes compared to normal tissue are used by pathologists as parameters for the classification and discrimination of tissues. Conventional histopathological analysis cannot be performed in situ or in real time since it requires previous tissue processing.

Cirrhosis is a general denomination for the final and irreversible stage of various chronic liver diseases of distinct etiologies, representing one of main causes of death not associated with neoplasias in the Western world. The more common etiologies of cirrhosis of the

liver are alcoholism, viral hepatitis and chronic changes of the biliary tree. Morphologically, cirrhosis of the liver is defined by the presence of fibrous septa that diffusely involve the liver and subdivide the parenchyma, with the formation of structurally abnormal regeneration nodules, complete alteration of architecture with spatial changes of the portal tract and hepatic veins and obliteration of small hepatic veins that are incorporated into venoportal septa. Its morphological diagnosis by means of a liver biopsy can be defined as diffuse scarring of the liver characterized by the loss of lobular architecture. Progressive fibrosis is the central characteristic of cirrhosis and, from a pathogenetic viewpoint, cirrhosis reflects the complex of superimposition of fibrosis and of hepatocyte regeneration due to inflammation and to chronic parenchymatous injury, which is the basis of most of the complications occurring in the last stages of chronic hepatic diseases. A consequence of this structural derangement is the inherent irreversibility of cirrhosis.

During the process of hepatic cirrhosis, fibrosis is represented by connective tissue that separates the liver into multiple nodules. The fibrous septa vary con-

siderably from delicate to extensive, often containing inflammatory cells and varying numbers of arterial, venous and biliary structures. In cases of well-established cirrhosis, fibrosis completely surrounds the nodules, although there are cases of incomplete septal cirrhosis with partial involvement of the regeneration nodules.

Regenerative hepatocyte hyperplasia is seen as an attempt to restore the integrity of the parenchyma, contributing to the formation of nodules and to the increased disorganization of cirrhosis. Despite its notoriety, the proliferative capacity of the hepatocytes is poorly understood and the mechanisms responsible have been intensively explored, with the regulation of normal hepatocyte growth being controlled by various growth factors.

The main complications of cirrhosis (hepatic insufficiency, portal hypertension and hepatocellular carcinoma) occur in the advanced and decompensated form of the condition.

Hepatocellular carcinoma (HCC) represents 80 to 90% of primary liver neoplasias and about 10% of systemic neoplasias. It occurs almost exclusively in patients with chronic liver diseases, in 90% of cases in livers in the phase of cirrhosis. The prevalence of the tumor in cirrhosis of the liver is sufficiently high to justify periodic and meticulous follow-up of cirrhotic patients. There are various mechanisms of liver carcinogenesis which are interrelated, synergic and still incompletely understood. They include specific processes such as indirect etiologic agent-hepatocyte interaction, genetic pathways as well as cirrhosis per se, a cyclic-progressive condition of inflammation-regeneration-fibrosis that includes dysplastic nodules. However, only 15 to 20% of patients with HCC benefit from potentially radical therapeutic options such as resection, liver transplantation and tumor alcoholization, with surgical indications being extremely judicious, especially in cirrhotic patients whose functional hepatic reserve is the main limiting factor regarding liver interventions.

Liver resection and transplantation currently represent the surgical treatments most frequently used, with safe benefits for patients with HCC. Hepatic resection is an operation whose success depends on the regenerative capacity of the liver. The methods described for patient selection are functional evaluation and tumor staging.

Evaluation and selection are necessary due to the reductions of the regenerative capacity of the liver

and to the degree of functional reserve typical of liver disease which, if not considered, may trigger signs and symptoms of postoperative hepatic insufficiency followed by multiple organ failure. Hepatic resection should be the minimum effective one corresponding to accepted oncology principles and should include the portal unit on which the tumor rests, this being one of the major pathways of tumor dissemination. Postoperative mortality is related to three factors: extension of resection, functional capacity of the remnant liver and serious vascular injuries.

Considering the standard techniques for the diagnosis and treatment of liver diseases and their limitations, there is great interest in the development of new technologies and of new methods to improve the current conditions detected. The new optic technologies have a high potential of incorporation into clinical principles of diagnosis and treatment in view of their principles of action and their operating characteristics. One of the most important properties is the possibility of selective interaction of laser light with a specific molecule or compound within a system full of other types of molecules, as is the case for biological tissues. The applications of optic techniques in medicine include: the use of laser as a surgical cutting and ablation instrument, phototherapy for the biomodulation of cell responses, optic diagnosis based on fluorescence and reactance, and photodynamic therapy for tumor treatment and microbial control. These techniques are being investigated and applied in different medical specialties. Fluorescence-based diagnosis is one of the main optic techniques for the detection of varied types of cancer. Laser-induced fluorescence of the target tissue investigated depends on the constituent biochemical and structural characteristics, with a normal tissue emitting a different fluorescence compared to neoplastic tissue. The fluorescence spectrum can be collected on the surface of the tumor and the information is obtained by a noninvasive and non-destructive procedure. After the determination of the spectral identities and of the appropriate processing for each type of disease investigated, in the specific case of the present project: cirrhosis, steatosis and HCC, the response can be obtained in real time. Experimental studies conducted on animal models by our group have demonstrated the sensitivity of the technique in discriminating between mild, moderate and severe steatosis and in the evaluation of the viability of the hepatic graft for transplant purposes. The spectral changes

observed in these two examples were confirmed by biochemical exams and by the evaluation of hepatic function, based on the amount of fat extracted from the liver in the case of steatosis, and by the evaluation of mitochondrial respiration at different times of graft investigation

Considerably attractive applications of optic diagnosis are: detection of HCC, cirrhosis, steatosis and metastatic lesions in the liver; delimitation of the margins of the lesions; evaluation of the viability of the hepatic graft without and with varying levels of steatosis; evaluation of hepatic ischemia and reperfusion; evaluation of the quality of cold perfusion and of reperfusion in livers to be transplanted.

Photodynamic therapy is a type of photochemotherapy based on the action of a photosensitizer, of light and of cellular oxygen. Tumor treatment is performed with a photosensitizer with selective interaction with neoplastic tissues which, after photoactivation, promotes the formation of highly reactive and oxidant compounds, the most important being singlet oxygen. As a response, the induction of tumor death mainly occurs by membrane lysis, damage to or inactivation of mitochondrial function, and vascular damage. The choice treatment for HCC is surgical resection, with the reestablishment of hepatic function depending on the quality and quantity of the remnant organ. In some cases hepatic resection is impossible due to the extension of the tumor which prevents the reestablishment of hepatic functions, with no treatment alternative. Photodynamic therapy may represent an alternative for tumor reduction, improving patient condition or even permitting a later resection of a reduced tumor mass. There still is no clinical protocol for photodynamic

therapy for the treatment of hepatic tumors. Studies, some of them still to be designed, will be conducted in order to determine therapy parameters such as form of administration and more adequate type of photosensitizer, interval between administration and illumination, and light dosimetry. Studies to be carried out in a rat model of HCC will emphasize the optic characterization of the tumor, the distribution and indirect quantitation of the photosensitizer, and induced necrosis as a function of different illumination parameters such as fluency (energy dose), irradiance, time of irradiation and light coupling geometry.

Both regarding hepatic resection and liver transplantation, in a pilot project we have performed functional hepatic evaluation. This evaluation has been performed by laser-induced fluorescence and based on the mitochondrial function of the liver in an attempt to assess the functional hepatic capacity of the liver remnant in cases of partial hepatic resection, and of liver vitality in transplants. For the diagnosis of hepatic tumors, from an experimental viewpoint it is necessary to induce experimental hepatocarcinomas in rats. Regarding the evaluation of hepatocellular function in liver transplantation, the study has been conducted on patients submitted to liver transplantation and their respective donors in the Clinical Transplant Program of the University Hospital, Faculty of Medicine of Ribeirão Preto, USP.

On this basis, reviews and viewpoints regarding the above topics are being prepared by several authors in order to be published, after due approval, in our journal in 2012, taking advantage of the process of regularization of publication of *Revista Medicina*, Ribeirão Preto.